$G\alpha_q$ signaling can lead to a greater understanding of these cardiovascular processes. G protein-coupled receptors activate $G\alpha_q$, and this allows $G\alpha_q$ to interact with its effectors, which include guanine nucleotide exchange factors (GEFs). Through formation of a complex with one of these effectors, p63RhoGEF, $G\alpha_q$ has been linked to activation of RhoA, a small molecular weight G protein and key actin cytoskeleton regulator. Although the atomic structure of the $G\alpha_q$-p63RhoGEF-RhoA complex is known, the mechanism of GEF activation is not clear, in part because the structure of the basal conformation of p63RhoGEF has not yet been determined. We are using nuclear magnetic resonance spectroscopy and small angle x-ray scattering to study the solution structure of the basal, inactive p63RhoGEF catalytic core and to confirm the multi-domain interactions with $G\alpha_q$ that were observed in the original crystal structure. $G\alpha_q$ signaling is also regulated by GTPase activating proteins known as regulator of G protein signaling (RGS) proteins. Whereas RGS2 is the only member of this family known to be selective for the $G\alpha_{q/11}$ class of heterotrimeric G proteins, multiple RGS family members are selective against $G\alpha_{q/11}$, and the molecular basis for this distinction is unknown. We have determined the crystal structure of $G\alpha_q$ in complex with RGS8, which is selective for multiple classes of $G\alpha$ subunits. This structure adds new insights into the molecular basis of RGS protein selectivity for distinct classes of $G\alpha$ subunits.

1729-Plat

Kinetics and Thermodynamics of Apicomplexa AMA1-RON2Sp Interaction

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Plasmodium falciparum and Toxoplama gondii are obligate intracellular protozoan parasites that invade and replicate within host cells. They both require the formation of a tight interaction with the host cell, called Moving Junctions (MJ), for successful infection. It has been shown that the MJ contains two key parasite components: the surface protein Apical Membrane Antigen 1 (AMA1) and its receptor, the Rhoptry Neck Protein (RON) complex, the latter one being targeted to the host cell membrane during invasion. Crystal structures of AMA1 proteins have shown a versatile loop, called domain II loop that extends into domain I likely to hide the RON2 binding site from host immunity. In the present work, we have studied association, dissociation reactions and binding equilibria of PfAMA1 and TgAMA1 reacting with their respective RON2 short peptide ligand. Equally, we have studied a deltaDII-loop-PfAMA1 construct to elucidate the role of this loop upon RON2 peptide binding. The reactions were tracked by fluorescence anisotropy as a function of temperature and concentration and globally fitted to acquire the rate constants to calculate the thermodynamic profile and propose a reaction mechanism. Our results showed that PfAMA1 and TgAMA1 bind to their respective RON2 peptide with the formation of one intermediate in a sequential reversible reaction: $A \leftrightarrow B \leftrightarrow C$. The reactions are both enthalpically and entropically favorable upon ligand binding thanks of the DII-loop induced fit folding down over the bound ligand forming a most stable final complex. The half life time of the complex at 25°C is 326s and 1077s for Pf and Tg complexes, respectively. By in vitro-in vivo extrapolation at 37°C, it is compatible with the time frame of erythrocyte invasion by Plasmodium falciparum merozoites. The elucidation of the binding mechanism brings new strategies for ligand discovery against these pharmacologically important targets.

1730-Plat

Conformation of the Troponin I C-Terminal Domain in Silico and in vitro: A Consideration of Dynamics in Comparing Simulation and Experiment

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The troponin complex acts as a molecular switch in striated muscle cells to regulate myosin attachment to and isomerization on actin filaments in response to changes in calcium concentration. Transitions between the inactive and active states of the thin filament require extensive domain movements and binding exchanges involving the C-terminal domain of cardiac troponin I (TnI-C), believed to be intrinsically disordered in the high-Calcium state [1]. Mutations in TnI-C are associated with hypertrophic cardiomyopathy, highlighting the importance of this domain in regulating cardiac contraction; however, the conformational flexibility of the domain has delayed its characterization compared with the rest of the troponin complex.

Here, we use single molecule Förster resonance energy transfer (smFRET) to probe the global conformation of TnI-C in the high-Calcium state, capitalizing on the technique's ability to analyze heterogeneous populations. We compare six pairwise distances within TnI-C to outputs from molecular dynamics simulations to gain insight into conformational sampling at finer detail and a faster timescale than experimental measurements allow. We find that simulations are in good agreement with smFRET measurements, but only after simulations are averaged over time. Simulations rarely, if ever, sample an ''ideal'' conformation matching all experimental measurements simultaneously; this finding highlights the importance of considering timescale when combining simulations and experimental measurements. Using our combined in silico and in vitro approach, we can predict areas of helical propensity and cluster potential global domain conformations in the unbound state, potentially providing mechanistic insight into the coupled binding and folding of this region during muscle relaxation.

1. Julien, O., Mercier, P., Allen, C.N., et al. (2011) Is there nascent structure in the intrinsically disordered region of troponin I? Proteins 79: 1240-1250.

1731-Plat

NMR Structural Studies of a 52 kDa Heterocylization Domain of the Yersiniabactin Non-Ribosomal Peptide Synthetase

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Nonribosomal peptide synthetases (NRPSs) are modular multi-domain enzymatic systems in bacteria and fungi that synthesize a diverse array of secondary metabolites called nonribosomal peptides (NRPs). NRPs encompass broad biological activity from etiological agents in microbial infections to various pharmaceutical applications. Despite their diversity NRPs are synthesized in a similar iterative manner, where each module of the synthetase adds a single substrate to the growing NRP chain. Chain elongation proceeds via peptide bond formation, catalyzed by condensation domains (C), between substrates tethered onto thiolation domains (T) in sequential modules. Condensation domains are sometimes replaced by cyclization domains (Cy) that carry out both condensation and heterocyclization (e.g. cysteines to thiazolines). NRP chain elongation affected by C (or Cy) and cognate T domain interactions is poorly understood due to lack of molecular details. Multidomain X-ray structures revealed only non-functional inter-domain orientations. Solution NMR studies have highlighted the presence of multiple conformers of excised domains in equilibria suggesting that transient domain interactions driven by conformational selection propel NRP chain elongation. Here, we investigate the molecular interactions between a Cy domain and its two cognate T domains from the NRPS Yersiniabactin synthetase using solution NMR techniques. We have determined the NMR solution structure of this excised 52 kDa Cy domain. New NMR methods were developed to overcome challenges in chemical shift assignments and improving the accuracy of distance constraints in the structure determination of this large protein. The interaction interfaces between the Cy and the T domains were mapped by chemical shift perturbation data from titrations of each T domain individually and sequentially into Cy. Our studies lay the foundation for building a model of this ternary complex to better understand domain interactions at a molecular level in NRP synthesis.

Platform: Bioengineering and Biomaterials

1732-Plat

Nanoparticle-Induced Membrane Pore Formation Studied with Lipid Bilayer Arrays

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Nanoparticles are present environmentally as byproducts of industrial processes and in a wide range of consumer goods. Although toxicity studies of nanoparticles and other nanomaterials have only begun relatively recently, a diverse range of nanoparticles has already been shown to be toxic. Nanoparticle cytotoxicity assays provide little information about the mechanisms of nanoparticle toxicity, which are potentially complex and are not likely the same for diverse nanoparticle species. For this reason, artificial lipid bilayer platforms have begun to be used as model systems for controlled studies allowing variation of experimental parameters not possible with cellular studies, such as membrane and solution composition. Unfortunately, in some cases the low throughput characteristic of lipid bilayer experimentation can limit the experimental scope. We have recently described a lipid bilayer array platform with simultaneous bilayer formation and measurement over a 32-element array with ~80% yield and no operator input following fluid addition.[1] The platform is modular and allows rapid cycling of the apparatus for

repeated measurement. We have used this platform to measure the interactions of aminated and carboxylated polystyrene nanoparticles and a range metal oxide nanoparticle species with lipid bilayers in a wide variety of experimental conditions, including nanoparticle concentration, bilayer composition, bilayer charge, presence of serum protein, solution ionic strength, and pH. The array format permitted several thousand bilayers to be measured in total with sufficient redundancy to give statistical significance to measured results. Detailed analysis of the electrical measurements shows pore formation that is dependent on electric field, ionic strength, and nanoparticle species.

1. ''Lipid bilayer arrays cyclically formed and measured,'' Bin Lu, Gayane Kocharyan and Jacob J. Schmidt, Biotechnology Journal 9, 446-451 (2014)

1733-Plat

Sticky Patches on Lipid Nanoparticles Generate Binding Geometries that Enable Effective Targeting of Otherwise Untargetable Cancers

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The majority of breast cancer patients (70%) have tumors designated as 'HER2-negative' (<1+ HER2-expression evaluated by immunohistochemistry or < 200,000 HER2-copies per cell). For these patients there are no targeted therapeutic options utilizing the HER2 receptor. The ability of conventionally targeted nanoparticles for specific targeting stops to hold on cancer cells expressing less than 200,000 copies of HER2 per cell or less than two receptors per nanoparticle's projected area (for particles of 100 nm in-diameter). This geometry corresponds to the limit of multivalent interactions (avidity) loosely defined as multiple contacts between neighboring same-cell receptors with ligands from a single nanoparticle. An alternative therapeutic approach is needed, therefore, to enable selective targeting and effective killing of cancer cells with low or too low HER2 expression.

Towards this goal we designed targeted lipid nanoparticles (vesicles) that contain HER2-targeting short peptides densely conjugated (for high local multivalency) within sticky patches. Sticky patches are phase-separated raft-like lipid-domains of high local multivalency which is induced by preferential partitioning of peptide-functionalized lipids. To enable selectivity in binding, sticky patches are exclusively triggered to form in mildly acidic environments matching the tumor interstitium. Lipid phase-separation with lowering pH is a result of the interplay of decreasing (pH-tunable) electrostatic repulsion and attractive hydrogen bonding among the domain-forming lipids.

We show that lipid nanoparticles with sticky patches selectively associate with and kill HER2-negative and triple negative breast cancer cells (MCF-7 and MDA-MB-231, respectively, with 60,000 and 90,000 HER2-copies per cell) while do not affect cardiomyocytes and breast normal cells. Systematic studies of association, dissociation and internalization rates of nanoparticles by cells will be presented, and a mechanistic mathematical model will be discussed with the aim to explain the observed high avidity.

1734-Plat

Controlled Activation of Protein Rotational Dynamics using Smart Hydrogel Tethering

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Stimulus-responsive hydrogel materials that stabilize and control protein dynamics have the potential to enable a range of applications that take advantage of the inherent specificity and catalytic efficiencies of proteins. Here we describe the modular construction of a hydrogel using an engineered calmodulin (CaM) within a polyethylene glycol (PEG) matrix that involves the reversible tethering of proteins through an engineered CaM-binding sequence. For these measurements, maltose binding protein (MBP) was isotopically labeled with [^{13}C] and [^{15}N], permitting dynamic structural measurements using TROSY-HSQC NMR spectroscopy. Protein dynamics are suppressed upon initial formation of hydrogels, with concomitant increases in protein stability. Relaxation of the hydrogel matrix following transient heating results in enhanced protein dynamics and resolution of substrate-induced large-amplitude domain rearrangements.

Our results demonstrate an ability to take advantage of the conformational sensitivities of hydrogel materials to activate protein dynamics upon transient temperature increases. Such an approach permits storage of proteins in an immobilized state prior to their activation, and contributes to important applications that can take advantage of the specificity of proteins for a range of sensing and chemical transformation applications. For example, single chain antibodies are shown to be dramatically stabilized against denaturation by urea, enabling their long term use for sensing applications. These smart materials possess optimized mass transfer properties (due to their high water content) and provide important avenues to detect ligands so as to link binding to material responses (e.g., proteolysis or other types of chemical transformation using the catalytic specificities of enzymes).

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1735-Plat

Immobilization of Proteins on Chemically Modified Germanium Investigated by ATR-FTIR

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The attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR) allows a detailed analysis of surface attached molecules, including their secondary structure, reaction mechanism, orientation and interaction with small molecules or proteins.[1] The aim of our study is the development of a universal immobilization technique on germanium for all kinds of proteins. We recently showed the specific immobilization of N-Ras and Photosystem I on a silane modified germanium surface.[1]

We now present a new approach employing thiol chemistry on germanium.[2,3] On one hand germanium crystals provide a great signal-to-noise ratio in ATR-FTIR. On the other hand protein immobilization via thiol chemistry is well-established because it is standard for modifications of gold surfaces e.g. in surface plasmon resonance. Here we combine the best of both worlds and report on germanium surface functionalization with different thiols which allowed for specific immobilization of histidine-tagged proteins with over 99% specific binding. The great advantage of using thiols in comparison with silanes is that a huge variety of thiols with functional groups for many kinds of protein immobilizations are readily available and the higher stability. Nativity of protein folding was confirmed by secondary structure analysis. Stimulus induced difference spectra were obtained for immobilized Channelrhodopsin 2, the small GTPase N-Ras and the phosphocholine-transferase AnkX, which demonstrated protein function at the atomic level.[4] Protein activity was observed for Channelrhodopsin 2 for over several days.[4]

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3: **Han, S**. *et al*. *J. Am. Chem. Soc*., 2001, 123, 2422-2425
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1736-Plat

Use of Short Amyloidogenic Peptides in Protein-Ligand Detection Systems

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Amyloid fibers, often associated with many human degenerative diseases (such as Alzheimer's and Parkinson's disease), may also have physiological roles, having even been suggested as potential novel biomaterials [1-2]. Since it is now clear that the amyloid fibers are much less toxic than their precursor aggregates [3], the interest for amyloidogenic species in nanosensing and protein-ligand detection sytsems increased dramatically [2]. Amyloid fibers in general share a common β-sheet rich architecture that is behind their exceptional stability, mechanical strength and resistance to degradation, which in nanotechnology makes them excellent nanomaterials candidates [1]. The potential to form amyloids (and other protein/peptide aggregates) can be predicted from the peptide amino acids sequence [1, 2]. Here, we used different amyloid peptide sequences to evaluate, by AFM, circular dichroism and FTIR spectroscopic approaches under different conditions, which type of amyloid species would be formed (namely amyloid oligomers, protofibrils or fibrils) at different times of incubation (24 hours, 72 hours and 2 weeks). AFM, CD and FTIR data taken together indicate that amyloid-based nanotechnology approaches may be successfully employed.

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